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14. ABSTRACT: Exposure to ionizing radiation at doses used in cancer radiotherapy and diagnostic radiology can increase breast cancer risk in women less than 45 years of age. Amifostine is a currently used cytoprotective agent. In addition to its cytoprotective effect, amifostine has been reported to inhibit radiation-induced mutagenesis in cultured cells and to protect against radiation-induced tumorigenesis in rodent model systems. This project is a pre-clinical study designed to determine if amifostine might be effective in preventing breast cancer initiation by medical exposures to ionizing radiation. The experiments will determine if amifostine is protective in a murine model of breast cancer initiation and, if so, determine the optimum dose, route and timing for its administration. The first year's objective was to test a high dose of amifostine administered I.P. prior to irradiation for reduction of ductal dysplasia in an outgrowth assay. Between September 2002 and May 2003, twenty-two donor mice were irradiated and 412 mammary fat pads were transplanted with mammary epithelial cells from these irradiated donors. Of these, 306 were harvested and examined as whole mounts. No dysplasias were seen, but due to the low outgrowth frequency no conclusions were drawn yet on the effectiveness of amifostine. In August 2003 the PI moved from M.D Anderson Cancer Center to Colorado State University and experienced a delay of nearly 2 years in having the grant transferred. During this time, breeding pairs of mice aged past their useful age. The project resumed in August 2005 with the establishment of a new breeding colony. Since that time, an additional 116 mice have been transplanted (232 fat pads) and outgrowths have been prepared for histology.					
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INTRODUCTION:

Historical medical exposures to radiation have been found to increase breast cancer risk. Some currently used diagnostic radiology and cancer radiotherapy procedures deliver doses to mammary tissue in susceptible girls and women that is comparable to the historical exposures. The research supported through DAMD17-02-1-0460 is designed to determine if a chemoprotective agent, amifostine, can prevent radiation-induced ductal dysplasias that progress to mammary tumors. The research design takes advantage of an inbred mouse strain that is susceptible to radiation-induced mammary tumors.

BODY:

The approved statement of work is:

Task 1. To determine if the frequency of radiation-induced ductal dysplasia in BALB/cBy J mice can be reduced by 400 mg/kg amifostine (Months 1-12):

- a. Mice required: 20 BALB/cByJ donors and 400 CB6F1 recipients. The recipients are F1 hybrids of BALB/c and C57BL/6J that are a bit more robust than BALB/c and give lower background levels in the dysplasia assay.

DONORS	TREATMENT	RECIPIENTS
10 BALB/c	saline	200 CB6F1
10 BALB/c	amifostine	200 CB6F1

- b. Methods: Assays on donor mice are set up weekly. In each assay a donor mouse will receive either saline or 400 mg/kg amifostine by intraperitoneal injection. Half an hour later the mouse will be irradiated to the whole body with 1 Gy in a ^{137}Cs small animal irradiator. After six weeks the donor will be sacrificed and the mammary epithelial cells will be harvested. The mammary epithelial cells will be transplanted into cleared mammary fat pads in 20 recipients (two fat pads per recipient). Ten weeks later the fat pads will be harvested from the recipients and processed as whole mounts. The whole mounts will be examined for ductal dysplasias and any suspected dysplasias will be confirmed by histological examination.
- c. The results of these experiments will indicate if a high dose of amifostine can reduce the frequency of radiation-induced ductal dysplasias in a susceptible mouse strain.
- d. Since amifostine has been shown to be active against radiation-induced tumorigenesis in other rodent models we anticipate that it will be effective in reducing radiation-induced ductal dysplasia in this system. However, if it is not we will screen other sensitive mouse strains (Weil in press) and other chemopreventive agents, including N-acetylcysteine and captopril .

Task 2. To determine if post-irradiation amifostine treatment can reduce the frequency of radiation-induced ductal dysplasia. If amifostine can be delivered post-irradiation it would be more acceptable as an agent to prevent radiotherapy-induced breast cancer, since it is less likely to provide tumor protection with this schedule of administration (Months 13-20):

a. Mice required: 15 donors and 300 recipients.

DONORS	AMIFOSTINE TREATMENT	RECIPIENTS
5 BALB/c	1/2 hour post-irradiation	100 CB6F1
5 BALB/c	2 hours post-irradiation	100 CB6F1
5 BALB/c	4 hours post-irradiation	100CB6F1

- b. Methods: As described in Task 1 except amifostine will be injected ½, 2 and 4 hours following irradiation.
- c. The results of these experiments will determine if amifostine can prevent ductal dysplasia if it is delivered after irradiation and, if so, provide a first indication of the how much later.
- d. We anticipate a simple positive or negative result. However, if no protection is seen we may also assay a time as short as 5 minutes post-irradiation.

Task 3. To determine if lower doses of amifostine are effective in preventing radiation-induced ductal dysplasias. Reducing the dose would decrease side effects and alleviate concern about tumor protection if the drug is administered during tumor radiathery (Months 21-30):

a. Mice required: 15 BALB/c donors and 300 CB6F1 recipients.

DONORS	TREATMENT	RECIPIENTS
5 BALB/c	100 mg/kg amifostine	100 CB6F1
5 BALB/c	20 mg/kg amifostine	100 CB6F1
5 BALB/c	1 mg/kg amifostine	100 CB6F1

- b. Methods: As described in *Task 1*, except decreasing doses of amifostine will be used.
- c. The results of these experiments will determine the lowest dose of amifostine, down to 1 mg/kg, that will reduce the frequency of radiation-induced ductal dysplasia.
- d. We do not anticipate problems with this task.

Task 4. Test oral administration of amifostine. Oral administration is easier than intravenous delivery in patients that are not receiving other intravenous medications. (Months 31-36)

a. Mice required: 5 donors 100 recipients.

- b. Methods: As described in *Task 1*, except amifostine will be delivered by gavage.
- c. The results of these experiments will determine if amifostine administered p.o. will reduce the frequency of radiation-induced ductal dysplasia.
- d. If oral administration is ineffective we will examine other routes such as subcutaneous or intramuscular injection. We will also test compounds similar to amifostine but more suited to oral administration such as WR-3689, WR151327, N-acetylcysteine and captopril.

The first task is to determine if high dose amifostine administered I.P. prevents radiation-induced ductal dysplasia. Work was begun at M.D. Anderson Cancer Center and transferred to Colorado State University. Transferring the grant required approximately two years. During that time mice used in the project were maintained on the Principal Investigator's start up funds until they became too old to use. A summary of the work to date, broken down by work site, is provided below.

M.D. Anderson Cancer Center

Treatment	Donors	Fat pads transplanted	Outgrowths	Dysplasias
Amifostine	6	148	39	0
none	6	158	34	0

The table reveals two shortcomings. The first is a technical matter, the low percentage of fat pads that have outgrowths. Ideally this number should be 70% but in these experiments it was only 24%. We have seen this before and other groups have also experienced similar reductions in outgrowth frequency. The decreased efficiencies have been transient and their cause(s) remain unknown. Although we had normal mammary epithelial cell yields from the donors and good cell viabilities we have, never the less, replaced all the reagents used in the assay. A second shortcoming is that only 412 transplants were made of the 800 proposed. Part of this was due to delays in setting up accounts and vendor shortages of BALB/cByJ mice.

Colorado State University

Treatment	Donors	Fat pads transplanted
Amifostine	14	92
none	22	140

All of the transplanted fat pads have been harvested and whole mounts prepared. They are currently being examined for ductal dysplasias.

KEY RESEARCH ACCOMPLISHMENTS:

- The ductal dysplasia assay is now up and running at Colorado State University

- 538 mammary fat pads have been transplanted with mammary epithelial cells from irradiated donors
- All transplanted fat pads glands have been harvested and prepared as whole mounts

REPORTABLE OUTCOMES:

None.

CONCLUSIONS:

The available outgrowths must now be examined to determine if amifostine treatment prior to irradiation reduces the frequency of ductal dysplasia. If so, we will initiate work on task 2.

REFERENCES:

None.

APPENDICES:

None.